

## INFLUENCE OF SEASONAL NITROGEN NUTRITION FLUCTUATIONS IN ORANGE AND LEMON TREES ON POPULATION DYNAMICS OF THE GLASSY-WINGED SHARPSHOOTER (*Homalodisca coagulata*)

J. L. BI,<sup>1,\*</sup> S. J. CASTLE,<sup>2</sup> F. J. BYRNE,<sup>1</sup> S. J. TUAN,<sup>1</sup> and N. C. TOSCANO<sup>1</sup>

<sup>1</sup>Department of Entomology, University of California, Riverside, CA 92521, USA

<sup>2</sup>Western Cotton Research Laboratory, USDA-ARS, Phoenix, AZ 85040, USA

(Received January 27, 2005; accepted June 15, 2005)

**Abstract**—The glassy-winged sharpshooter (GWSS) is a xylem feeder that develops conspicuous, year-round populations in many citrus-producing regions of California. Field studies were conducted in a combined lemon and orange orchard to determine the influence of changing amino acid concentrations on relative densities of GWSS. Nineteen protein amino acids were detected in xylem fluid of both lemon and orange trees. Although annual profiles of total and essential amino acids were similar for each citrus species, mean concentrations were consistently higher in lemons than in oranges for most of the year, except for one critical period in late winter. Yearly spring when concentrations were higher in oranges. Principal component analysis followed by factor analysis was performed individually on lemon and orange data sets to identify a reduced number of orthogonal factors composed of amino acids having similar seasonal profiles. Four factors were identified for each citrus species that accounted for 85% and 79% of the total variation in the orange and lemon analyses, respectively. These were then examined with respect to shifts in GWSS numbers that occurred asynchronously in lemons and oranges over the annual population cycle. Three distinctive number shifts were identified that included a peak in adult numbers in lemons during August 2001, significantly higher numbers in lemons relative to oranges during midwinter, and finally an increase in oranges of both adults and nymphs during spring 2002. Various groups of amino acids, i.e., factors, displayed peak annual or elevated levels during the intervals when shifts in GWSS numbers were occurring. Soluble protein levels in oranges and lemons did not correspond to shifting GWSS numbers as certain amino acids. However,

\* To whom correspondence should be addressed. E-mail: jianbi@citrus.ucr.edu

soluble protein levels were higher in oranges during late winter/early spring when GWSS adults were sexually active. Potential roles of these amino acids and proteins in GWSS host selection are discussed.

**Key Words** *Homalodisca coagulata*, GWSS numbers, citrus, free amino acids, soluble proteins, citrus-GWSS interactions.

## INTRODUCTION

The glassy-winged sharpshooter (GWSS) *Homalodisca coagulata* (Say) is an exotic insect in California and important vector of *Xylella fastidiosa*, a xylem-limited, plant pathogenic bacterium that causes Pierce's disease (PD) in grapes (Purcell, 1981; Blua et al., 1999). Although it is unknown when and how GWSS first arrived in the state, the earliest recorded specimen was collected in 1989 near Irvine (Sorensen and Gill, 1996). During the 1990s, populations expanded throughout much of southern California and became conspicuous in citrus orchards and vineyards as well as in much of the urban landscape (Blua et al., 1999). In Temecula valley, a major wine grape production area in southern California, wineries have lost 20Y30% of their vines to PD since 1997 (Hix, 2001). Although the disease has been in the state since the 1880s and outbreaks have occurred periodically over the last century, PD was apparently limited by the fact that native sharpshooters do not fly far from their preferred native habitats to develop large populations (Varela, 1996). With the introduction of the GWSS, which flies farther and feeds on a greater range of plants than California's native sharpshooters, PD problems in vineyards have been increasing dramatically (Blua et al., 1999). The threat from the GWSS is not limited to the grape industry. Since becoming established in California, the GWSS has also been responsible for vectoring oleander leaf scorch and almond leaf scorch diseases (Blua et al., 1999; Almeida and Purcell, 2003). In addition, citrus growers have been hampered by restrictions upon shipments of citrus fruits across county lines imposed by the State of California in an attempt to prevent spread of GWSS to new areas of the state. Less certain at this time is the potential impact on fruit quality and yield in some citrus orchards that have sustained heavy infestations of GWSS over the past decade.

GWSS is highly polyphagous and may feed on over 100 plant species (Turner and Pollard, 1959; Adlerz, 1980). One of the most important hosts in California is citrus and the proximity of citrus groves to vineyards has influenced the incidence and severity of PD in grapes (Perring et al., 2001). It is imperative that effective control strategies be implemented to curb the spread of the vector. Vital to this effort would be establishing the host plant range of the GWSS and determining the physiological and biochemical mechanisms for host selection.

Dietary nitrogen is an important nutritional index impacting survival, growth, and reproduction of phytophagous insects (White, 1984; Bi et al., 1994, 1997, 2001, 2003; Simpson et al., 1995; Joern and Behmer, 1997; Blackmer and Byrne, 1999). Nitrogen nutrients are particularly limited for xylophagous insects, such as GWSS, because xylem fluid consists of over 95% water and is the most dilute food source for herbivores (Anderson et al., 1989, 1992). The primary nitrogen nutrients in xylem fluid are amino acids and soluble proteins (Anderson et al., 1989, 1992; Bi and Toscano, unpublished data). There are 19 amino acids detectable in the xylem fluid of most host plants, and amides (glutamine and asparagine) are predominant in most woody host plant species investigated (e.g., Anderson et al., 1989, 1992; Brodbeck et al., 1993, 1996, 1999). Adult GWSS prefers and performs best on host plants containing high contents of amides in the xylem fluid (Brodbeck et al., 1990; Anderson et al., 1992), whereas immatures require lower levels of amides and higher levels of many other amino acids (Brodbeck et al., 1996, 1999). Most of those results were derived from studies of GWSS interaction with grape, soybean, peach, or some wild host plant species (e.g., Anderson et al., 2003; Brodbeck et al., 2004).

GWSS host selection and utilization among citrus plant species and the related nutritional mechanisms have not been reported. As a year-round host in southern California, citrus plays a critical role in the feeding and reproductive ecology of GWSS. The present research was initiated to determine the influence of seasonal nitrogen nutrition fluctuations in xylem fluid on GWSS population dynamics on lemon and orange trees.

#### METHODS AND MATERIALS

*Experimental Plots.* A lemon and orange mix-planted orchard at the Agricultural Experimental Station, University of California, Riverside was used for the experiment. Three blocks of 30 orange (*Citrus orange* L. var. Frost Valencia grafted on Troyer Citrange) and 30 lemon (*C. lemon* L. var. Lupe grafted on Cook) trees adjacent to one another were used. Both orange and lemon trees were 30 yr old. Five trees of lemon or orange were randomly selected from each block to monitor the GWSS population dynamics and to extract xylem fluid.

*Collection of Xylem Fluid.* Xylem fluid was used for determination of nutritional quality because GWSS exclusively feeds on xylem fluid (Anderson et al., 1989). Collections of xylem fluid were done between 6:00 A.M. and 10:00 A.M. on biweekly sampling dates using a pressure bomb apparatus (Anderson et al., 1989, 1992). One to three terminal shoots from each tree were used for xylem fluid extraction because these shoots are the preferred feeding sites for GWSS on citrus plants (Mizell and French, 1987). Upon collection, the xylem fluid was immediately placed on dry ice before final storage in a  $-80^{\circ}\text{C}$  freezer.

The samples were used for chemical analyses of free amino acids and soluble proteins. Sampling was initiated on June 8, 2001 and terminated on May 30, 2002.

*Determination of Free Amino Acids and Soluble Proteins.* Free amino acids were quantified with a Perkin Elmer Applied Biosystem Model 420A PTC derivatizer with an on-line Perkin Elmer Applied Biosystem Model 130A PTC Amino Acid Analyzer. After filtration with a 0.45- $\mu$ m syringe filter, 20  $\mu$ l of xylem fluid were reacted with phenylisothiocyanate (PITC) to produce phenylthiocarbamyl amino acid derivatives. Following derivatization, a methanol solution containing the PTC amino acids was transferred to an HPLC system for separation. PTC amino acids were separated on a Spheri-5 PTC column (220  $\times$  2.1 mm), and the PTC chromophore was detected at 263 nm. The buffer system used for separation was 50 mM sodium acetate pH 5.45 as buffer A and 70% acetonitrile/32 mM sodium phosphate pH 6.1 as buffer B. The program was run using a gradient of buffer A and buffer B with an initial 7% buffer B concentration and ending with a 60% buffer B concentration at the end of the gradient. A standard mixture of known quantities of individual amino acids (Sigma) was derivatized as described above and used for quantification and identification of individual amino acids.

Protein content was determined by the Bradford method (Bradford, 1976). Three 20- $\mu$ l aliquots of each sample were mixed with 180  $\mu$ l of Bio-Rad (Bio-Rad, Richmond, CA, USA) protein assay reagent. Absorbance of the reaction mixture was read at 595 nm with a spectrophotometer, and protein content was determined from a standard curve established using known quantities of bovine serum albumin (from Sigma) and the above reagent.

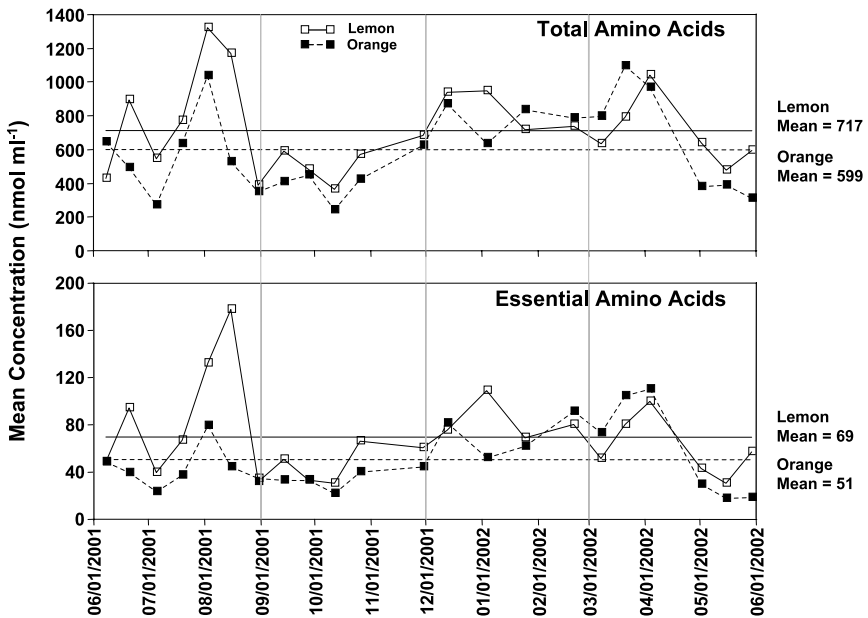
*Sampling of GWSS.* A bucket-sampling device (5-gal plastic bucket, with a funneling collector in the bottom, attached to an extendable pole), as described by Castle et al. (2005), was used to sample both immature and adult GWSS in orange and lemon trees. Seven beats were processed with the bucket in each of the five directions (360°/5) around the trees. Numbers of adults and nymphs in the collector from each tree were then counted. Population dynamics of both adult and immature GWSS were monitored on a weekly basis. The same 15 trees used for xylem fluid extraction from each citrus species were used for monitoring GWSS population dynamics.

*Statistical Analyses.* Multivariate statistical methods were used to identify groups of amino acids that exhibited similar dynamical behavior. Principal component analysis followed by factor analysis (SAS Institute Inc., 1989) was run separately for lemon and orange data sets. Outlier analysis using Mahalanobis distances was applied prior to factor analysis to eliminate outlying points from the analysis. Individual amino acids with loading values  $>|0.6|$  within each factor were grouped and related to shifts in GWSS adult populations as characterized by increasing numbers in one citrus species while remaining static or decreasing in the other. Analysis of variance (ANOVA) and the least significant

difference (LSD) test in one-way randomized complete block general linear models (GLM) (SAS Institute Inc., 1989) were used to analyze the data and separate the means of protein data in each sampling date. A repeated-measures analysis of variance (SAS Institute Inc., 1989) was applied to time segments in which visible shifts in GWSS numbers were apparent by graphical analysis. Numbers of GWSS adults and nymphs from bucket-collected samples were  $\log(y + 1)$ -transformed before ANOVA to normalize the data (Little and Hill, 1978).

RESULTS

*Free Amino Acids and Soluble Proteins.* Annual amino acid profiles were similar for each citrus species, but levels were consistently higher in lemons than oranges for much of the year, save for one critical period in late winter. Yearly spring when concentrations were higher in oranges (Figure 1). Levels of total amino acids fluctuated by >3-fold for lemons and >4-fold for





profile for each species with higher levels in oranges occurring notably during the late winter/early spring period (Figure 1). Overall, however, the annual mean level of essential amino acids in lemons (69 nmol/ml) was higher than in oranges (51 nmol/ml).

Nineteen protein amino acids were detected in both lemon and orange xylem fluid samples. The proportion of total amino acids made up by individual amino acids varied more than 3 orders of magnitude in lemons and oranges. By far, the highest level of any amino acids was proline, varying between 52% and 62% of the total depending on species and time of year (Figure 2a, b). The mean level of asparagine was the next highest in both species during three out of four seasons, accounting for 7Y17% of the total. Other amino acids in relative high levels were glutamine (1Y12% of the total), serine (2Y9%), and aspartate (1Y6%). The rest of amino acids were less than 5% of the total. Mean levels for

TABLE 1. FACTOR LOADING VALUES FOR AMINO ACIDS IN XYLEM FLUID FROM ORANGE AND LEMON TREES COLLECTED OVER A 1-YR PERIOD

Amino acids	Factor							
	Orange				Lemon			
	1	2	3	4	1	2	3	4
Aspartic acid	-0.390	<b>0.836<sup>a</sup></b>	-0.003	0.073	0.099	<b>0.826</b>	0.350	-0.143
Glutamic acid	-0.218	<b>0.805</b>	0.140	0.193	0.223	<b>0.872</b>	0.023	0.070
Asparagine	<b>-0.819</b>	0.283	0.203	0.019	0.375	0.259	-0.113	-0.600
Serine	-0.410	<b>0.822</b>	-0.005	-0.009	0.496	<b>0.695</b>	0.182	-0.269
Glutamine	<b>-0.870</b>	0.241	0.045	0.015	<b>0.899</b>	0.126	-0.154	-0.288
Glycine	<b>-0.617</b>	0.481	-0.017	0.153	-0.002	0.350	<b>0.715</b>	-0.233
Histidine	<b>-0.911</b>	0.262	0.087	0.082	<b>0.613</b>	0.084	0.270	-0.558
Arginine	0.022	0.200	-0.009	<b>0.941</b>	-0.059	<b>0.742</b>	-0.076	-0.328
Threonine	<b>-0.840</b>	0.447	0.079	-0.080	<b>0.757</b>	0.265	0.089	-0.487
Alanine	-0.187	<b>0.811</b>	-0.049	0.116	0.008	<b>0.0760</b>	-0.054	-0.419
Proline	-0.554	0.505	-0.100	0.431	<b>0.619</b>	0.448	0.390	-0.281
Tyrosine	<b>-0.860</b>	0.402	-0.086	-0.041	0.181	0.240	0.054	<b>-0.779</b>
Valine	<b>-0.950</b>	0.248	0.052	0.004	<b>0.647</b>	0.017	0.263	<b>-0.624</b>
Methionine	<b>-0.832</b>	0.036	-0.221	0.214	<b>0.901</b>	0.053	0.123	-0.049
Cysteine	-0.014	-0.032	<b>-0.954</b>	0.019	0.126	-0.103	<b>0.708</b>	-0.001
Isoleucine	<b>-0.949</b>	0.238	0.024	-0.009	<b>0.795</b>	0.019	0.276	-0.476
Leucine	<b>-0.863</b>	0.374	-0.156	-0.014	0.365	0.181	0.527	<b>-0.654</b>
Phenylalanine	<b>-0.870</b>	0.307	-0.222	0.027	0.397	0.331	0.206	<b>-0.718</b>
Lysine	<b>-0.881</b>	0.339	0.039	0.096	0.436	0.301	0.417	<b>-0.651</b>
Eigenvalue	12.089	1.982	1.187	0.910	0.920	2.579	1.443	1.077
Percent	63.6	10.4	6.2	4.8	52.2	13.6	7.6	5.7
Cumulative %	63.6	74.1	80.3	85.1	52.2	65.8	73.4	79.0

<sup>a</sup> Loading values > |0.6| are in bold typeface.

individual amino acids ranged 10-fold or greater both within and among trees over each season (Figure 2a, b). Seasonal mean levels for all amino acids (exclusive of proline) varied by 1.8- and 2.2-fold for lemons and oranges, respectively, with fall representing the lowest mean concentrations for both species. Thus, less variation in mean levels of all amino acids was observed among seasons compared to the 3- to 4-fold shifts that occurred within particular seasons.

Distinctive groups of amino acids between lemons and oranges were apparent following factor analysis. Separate analysis on each species produced a rotated loading matrix that had four factors (eigenvalues  $>1$ ) for lemons and three factors for oranges, but a fourth factor was subsequently included for orange based on an eigenvalue close to 1 (0.91) (Table 1). Over 63% of the variance in the solution for oranges was accounted for by factor 1 that contained 12 variables (asparagine, glutamine, glycine, histidine, threonine, tyrosine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine) with loading values  $>|0.6|$ , but all the amino acids with the exception of glycine having values  $>|0.8|$ . Factor 2 accounted for 10.4% of the solution for orange and contained four variables (aspartic acid, glutamic acid, serine, and alanine) with loading values  $>|0.6|$  (Table 1). A single variable (cysteine or arginine) was accounted for in factors 3 and 4 for oranges, bringing the total number of amino acids grouped by factor analysis to 18, the lone exception being proline. The cumulative variance explained by the solution for orange was 85.1% compared to 79% explained by the four factors in the lemon solution. Only seven amino acids (glutamine, histidine, threonine, proline, valine, methionine, and isoleucine) met the criterion for factor 1 in the lemon solution, but at a lower level of

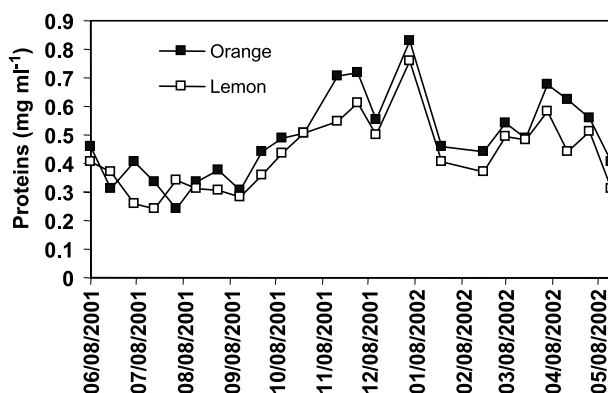


FIG. 3. Mean concentrations of soluble proteins from June 2001 through May 2002 in lemons and oranges.



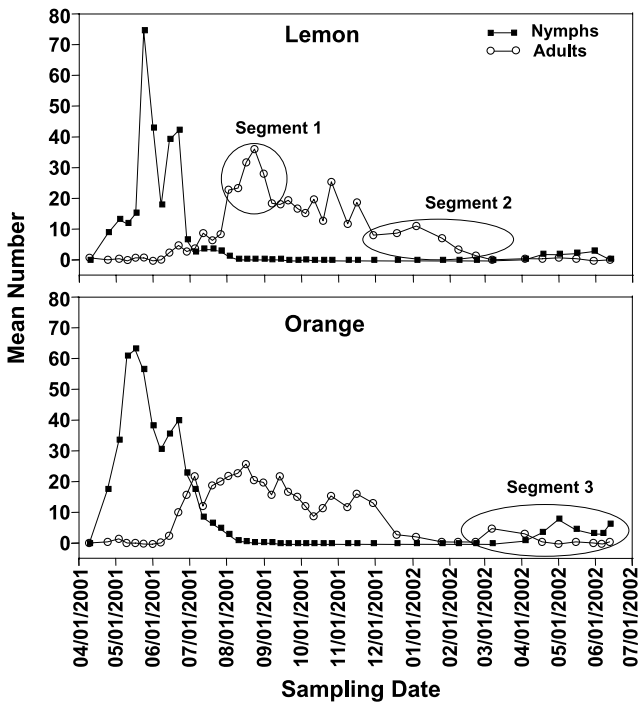


FIG. 4. Mean numbers of GWSS nymphs and adults in lemons (top) and oranges (bottom) between April 2001 and June 2002. Encircled areas in each chart are examined in greater detail in subsequent figures.

correlation than observed for factor 1 in the orange solution. Factor 2 for lemons contained all four amino acids that grouped in factor 2 for oranges, but also included alanine (Table 1). Factor 3 contained two amino acids (glycine and cysteine), but factor 4 for lemons contained six amino acids (asparagine, tyrosine, valine, leucine, phenylalanine, and lysine) compared to one for oranges. Only one amino acid (valine) in both analyses correlated with more than one factor (factors 1 and 4) in the lemon analysis.

Soluble protein contents in orange and lemon xylem fluid samples ( $P > 0.05$ ) were similar throughout much of the experimental period with the exception of five sampling dates (September 28, 2001; November 17 and 30, 2001; April 18, 2002; and May 30, 2002, respectively) when the content was 18Y40% higher ( $P < 0.05$ ) in orange xylem fluid than in lemon xylem samples (Figure 3).

*GWSS Numbers and Their Responses to the Nitrogen Nutrients.* The annual profiles of GWSS nymphs and adults are quite similar for lemons and oranges

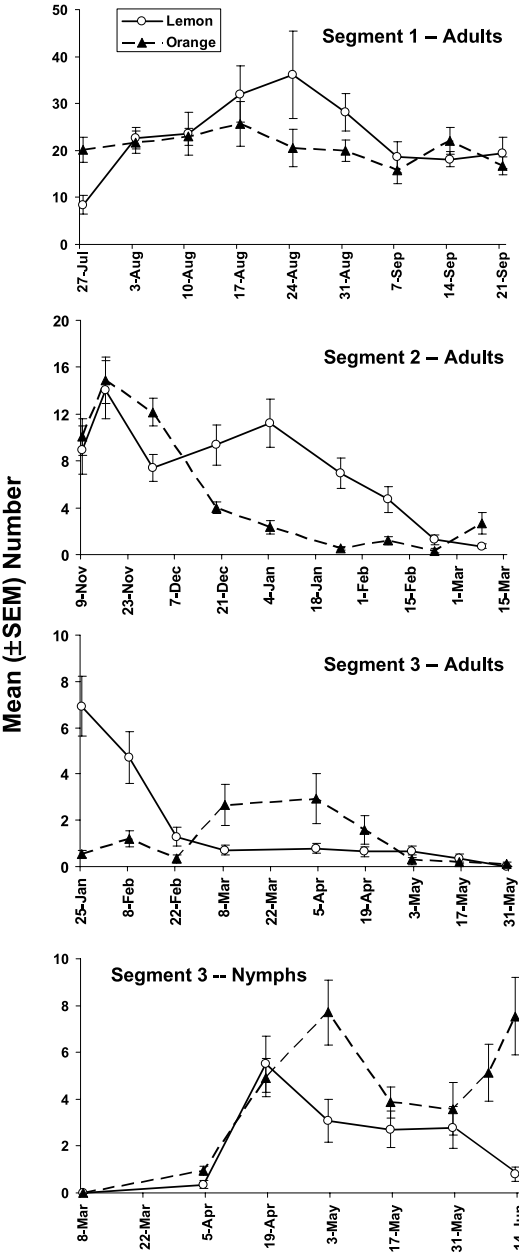


FIG. 5. Comparative numbers of GWSS adults and nymphs in lemons and oranges at three different periods during the annual cycle (see Fig. 4).

(Figure 4), but with notable fluctuations in GWSS numbers occurring at specific times on either lemons or oranges. We identified three intervals during the annual cycle that GWSS numbers increased on one species while remaining static or declining in the other. Two of these intervals occurred as increases in GWSS adults in lemons (Figure 4) while the third interval occurred with both adults and nymphs increasing in oranges (Figure 4).

To examine the changes in numbers on lemons or oranges more closely, the circled and numbered areas in Figure 4 were expanded to provide greater detail on GWSS numerical differences between lemons and oranges and to focus attention on near-synchronous changes that occurred in certain amino acids. The first departure of relative numbers of adult GWSS occurred on July 27–September 7, 2001 (Figure 5, Segment 1). Mean adult numbers on lemon trees had been depressed over three consecutive sampling dates from July 13 through July 27, but by August 3 had more than doubled to a level equivalent to the numbers on oranges (Figure 5, Segment 1). One wk later (August 10), adult numbers were again equal on lemons and oranges, but then increased over the next 2 wk on lemons while remaining fairly constant on oranges. After peaking on August 24, adult numbers on lemons and oranges declined over the next 2 wk with near parity between the two citrus species on September 7. A repeated-measures ANOVA conducted on the data from the seven sampling dates between July 27 and September 7 yielded a nearly significant result ( $F_{6,12} = 2.86$ ,  $P = 0.057$ ) of higher adult GWSS numbers in lemons than oranges. In the second example, adult numbers in lemons were significantly greater ( $F_{5,13} = 6.03$ ,  $P = 0.004$ ) than in oranges during the period November 30, 2001–February 22, 2002 (Figure 5, Segment 2). Adult numbers in oranges dropped quite drastically after November 30, 2001, while remaining stable in lemons and even gaining slightly (Figure 5, Segment 2). By early spring 2002, few GWSS adults were present on lemons or oranges, their numbers apparently having declined due to winter mortality. However, this is a critical time of the year as adults become sexually active and soon begin laying egg clutches that eventually emerge as adults beginning in June each year. A significant shift ( $F_{4,33} = 3.03$ ,  $P = 0.031$ ) in GWSS adult numbers to oranges from lemons occurred between February 22 and May 2, 2002 (Figure 5, Segment 3V Adults). Higher adult numbers in oranges during early spring translated into significantly higher nymphal numbers ( $F_{4,33} = 2.98$ ,  $P = 0.033$ ) in oranges between April 18 and June 13 (Figure 5, Segment 3V Nymphs).

The peak in GWSS adults reached on August 24 (Figure 5, Segment 1) corresponds closely to the rise and fall in levels of 10 amino acids that were grouped into two different factors in the lemon factor analysis (Table 1). The five amino acids that grouped into factor 2, including aspartic acid, glutamic acid, serine, alanine, and arginine (Figure 6a), had only one significant peak over the entire year that occurred in August. In contrast, the five amino acids grouped into factor 4, including tyrosine, lysine, asparagine, phenylalanine, and

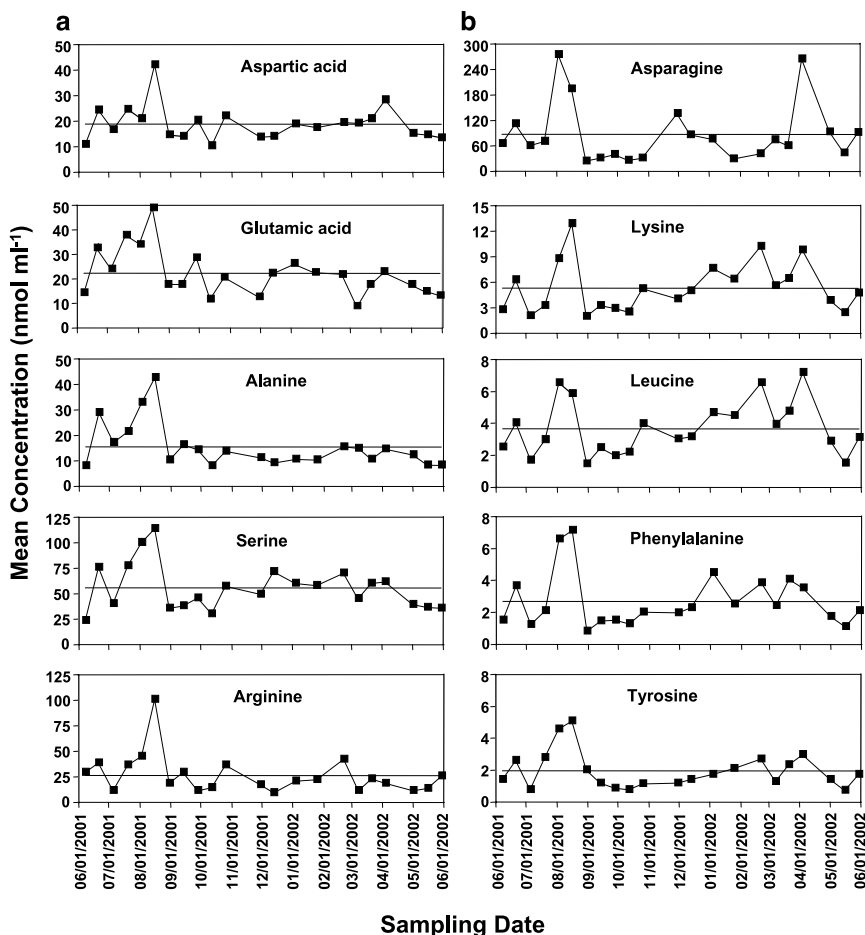
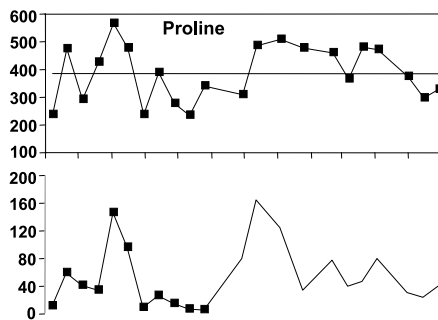


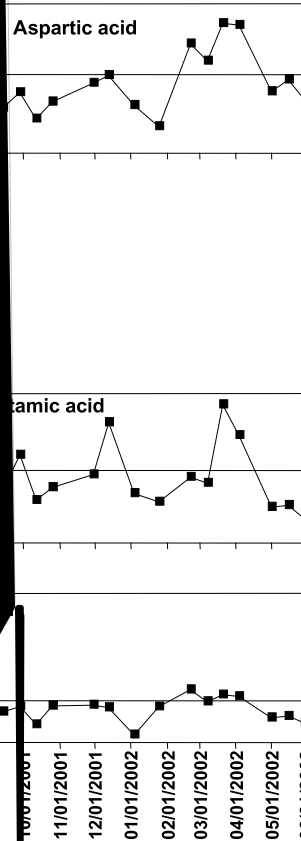
FIG. 6. Annual profiles of five amino acids that loaded onto (a) factor 2 in the lemon analysis, and another five amino acids that loaded onto (b) factor 4. The horizontal line in each chart is the annual mean concentration.

leucine (Figure 6b), had their highest peaks in August, but also showed secondary peaks in levels during the following spring. In addition to these 10 amino acids, six out of the seven amino acids that grouped into factor 1, including threonine, valine, isoleucine, glutamine, proline, and histidine (Figure 7), also displayed an early August peak in their levels that likely contributed to the increase in numbers of GWSS adults recorded on lemons relative to oranges.

A more critical role for the seven amino acids grouped in factor 1 of the lemon analysis (Figure 7) may have occurred in midwinter at a time that GWSS







numbers in lemons consistently exceeded those in oranges (Figure 5, Segment 2). The levels of threonine, valine, histidine, and isoleucine all reached peaks on January 4, 2002 during this interval. Methionine and glutamine were also at near peak levels during this interval, reaching their peak levels one date earlier on December 19, 2001. In fact, annual peaks were attained for each of these amino acids with the exception of valine and threonine, whose concentrations during this period were just slightly below annual peak levels.

Amino acid concentrations in oranges also showed fluctuations from season to season, but on a different time frame than lemons. The most coherent group of amino acids in either the lemon or orange factor analyses occurred in

the orange analysis with 12 amino acids grouped into factor 1. These include valine, leucine, isoleucine, histidine, threonine, lysine, tyrosine, asparagine, glutamine, methionine, phenylalanine, and glycine (Figure 8a). Concurrent with the early spring peak in these 12 amino acids was a shift in GWSS adults (Figure 5, Segment 3V Adults) from lemons to oranges that subsequently resulted in a greater number of nymphs on orange (Figure 5, Segment 3V Nymphs). The levels of all 12 amino acids in factor 1 of the orange analysis had significantly increased by February 22, 2002, with most reaching their annual peak levels in late March or early April. This closely corresponds to the time in late winter and early spring when GWSS adults are reproductively active as evidenced by pairs in copula and the presence of brochosomes on adult females. In addition to the 12 amino acids in factor 1, three out of the four amino acids that grouped into factor 2 of the orange analysis also displayed peak levels during spring (Figure 8b). However, these three amino acids, aspartic acid, glutamic acid, and serine, also had other periods of peak levels during the year in contrast to the 12 amino acids in factor 1 that displayed a prolonged rise in levels peaking in early spring.

## DISCUSSION

Throughout the experimental season, 19 protein amino acids were detected in both lemon and orange xylem fluid in this study. These results are consistent with previous reports on amino acids in xylem fluid of GWSS hosts such as *Baccharis halimifolia* (L.), *Lagerstroemia indica* (L.), *Prunus salicina* (Lindl.), *Prunus persica* (L.), *Pyrus communis* (L.), *Vitis hybrid*, *Catharanthus roseus* (L.), and *Glycine max* (L.) (Brodbeck et al., 1990, 1993, 1999, 2004; Anderson et al., 1992). The predominant amino acids detected in this study were proline and asparagine. Our results agree with a previous report in which proline was the most abundant amino acid in *C. sinensis* (L.) (Moreno and Garcia-Martinez, 1983). In contrast, asparagine and glutamine were predominant (over 50% of the total) in other GWSS hosts (Brodbeck et al., 1990, 1993, 1999, 2004; Anderson et al., 1992). Apparently, those differences may be due to the differences in host plant families and/or species.

Although GWSS is capable of feeding on over 100 plant species, well-defined host preferences vary seasonally and successful development often requires host switching (Mizell and French, 1987; Brodbeck et al., 1999; Redak et al., 2004). In many instances, host switching may represent multiway traffic as GWSS adults are highly mobile and often conspicuous in their movements to and from among various hosts. At times, however, population levels on particular host species are apparently higher over the others, presumably due to nutritional requirements being better satisfied by these host species. In the present study, only the two citrus species were available as hosts to the GWSS



population as the study area was centrally located within a 12-ha orchard split evenly between lemons and oranges. Despite the close relationship between lemon and orange and the apparent similarity in annual amino acid profiles (Figure 1), there were clear instances when the GWSS population was more concentrated in one species than the other (Figure 5). Factor analysis of each data set, lemon and orange, revealed different groups of amino acids that varied from one another in terms of their seasonal peak concentrations as well as the number of peaks they exhibited over the annual cycle. By aligning the three distinctive intervals of host switching by GWSS between lemons and oranges with amino acid groups from the lemon and orange factor analyses, it was possible to infer nutritional shifts that were occurring concomitantly to the GWSS population shifts.

This study revealed that adult GWSS numbers responded positively to higher levels of aspartic acid, glutamic acid, serine, alanine, asparagine, tyrosine, lysine, asparagine, phenylalanine, leucine, methionine, threonine, valine, histidine, isoleucine, glutamine, and proline in xylem fluid of lemons, and valine, isoleucine, threonine, tyrosine, glutamine, phenylalanine, leucine, histidine, lysine, asparagine, methionine, glycine, aspartic acid, glutamic acid, and serine in xylem fluid of oranges. These amino acids may serve as important nutrients to determine host preference for GWSS. Indeed, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine, arginine, and histidine are considered as essential amino acids for insect growth and development (Taylor and Medici, 1966; Brodbeck and Strong, 1987). In previous studies, the levels of asparagine and glutamine in xylem fluid of four host species (*L. indica*, *B. halimifolia*, *P. persica*, and *P. salinicia*) have been positively correlated with GWSS host selection (Brodbeck et al., 1990). The fact that glutamine and asparagine have high nitrogen/carbon ratios and account for high caloric value may make them an ideal host selection cue for generalist feeder GWSS (Brodbeck et al., 1990), especially adult GWSS in their sexually active status. Both of these amides are also considered phagostimulants for GWSS (Anderson et al., 1992). It is noted that adult GWSS females produce protein-containing brochosomes just prior to egg laying (Hix, 2001). Higher levels of these amino acids may be critical for the brochosome production.

Previous research indicated that adult GWSS prefers and performs best on host plants containing high contents of amides in the xylem fluid (Brodbeck et al., 1990; Anderson et al., 1992), while immatures require lower levels of amides and higher levels of many other amino acids, thus the more balanced amino acid profiles (Brodbeck et al., 1995, 1996, 1999). Our results showed that a majority of GWSS eggs are laid on oranges, and the immatures then grow and fully develop from April to July on oranges when amino acid (especially glutamine and asparagine, the second and third most predominant amino acids in the xylem fluid) fluctuations drop to minimal level to make the profiles more

balanced. During this period, levels of isoleucine, tyrosine, phenylalanine, and lysine are particularly higher in oranges than in lemons. It is known that tyrosine and phenylalanine are essential for insect cuticle formation (Bernays and Woodhead, 1984). Thus, immature GWSS may need higher levels of these two amino acids for their successful development of cuticle.

Increased protein quantity is frequently associated with enhanced insect survival, growth, and fecundity (McNeill and Southwood, 1978; Mattson, 1980; Ohgushi, 1992; Slansky, 1993). Protein quality also affects insect performance (Bi et al., 1994; Bi and Felton, 1995; Felton, 1996; Bi et al., 1997). Our results indicated that substantial amounts of soluble proteins exist in xylem fluid of GWSS host plants. Soluble protein levels in xylem fluid of oranges and lemons were similar except on a few of the sampling dates (e.g., mid-April) when levels were significantly higher in xylem fluid of oranges than in lemons. Although it is unknown how GWSS utilizes host proteins, soluble proteins may help GWSS female to produce brochosomes for the egg laying (Hix, 2001). Our results clearly indicated that the soluble protein levels in April and May were higher in oranges. The effect of protein quality in host xylem fluid on GWSS performance and host selection warrants further investigation.

*Acknowledgments* We thank Dr. Tim Kingan and Mr. Gerald Porter for their technical support. This research was funded in part by the University of California Pierce's Disease Control Program.

## REFERENCES

- ADLERZ, W. C. 1980. Ecological observations on two leafhoppers that transmit the Pierce's disease bacteria. *Proc. Fla. State Hort. Soc.* 93:115Y120.
- ALMEIDA, R. P. P. and PURCELL, A. H. 2003. Transmission of *Xylella fastidiosa* to grapevines by *Homalodisca coagulata* (Hemiptera: Cicadellidae). *J. Econ. Entomol.* 96:264Y271.
- ANDERSON, P. C. BRODBECK, B. V. and MIZELL, R. F. III. 1989. Metabolism of amino acids, organic acids, and sugars extracted from the xylem fluid of four host plants by adult *Homalodisca coagulata*. *Entomol. Exp. Appl.* 50:149Y160.
- ANDERSON, P. C. BRODBECK, B. V. and MIZELL, R. F. III. 1992. Feeding by the leafhopper, *Homalodisca coagulata*, in relation to xylem fluid chemistry and tension. Feeding by the leafhopper, *Homalodisca coagulata*, in relation to xylem fluid chemistry and tension. *J. Insect Physiol.* 38:611Y622.
- ANDERSON, P. C. BRODBECK, B. V. and MIZELL, R. F. III. 2003. Plant and insect characteristics in response to increasing density of *Homalodisca coagulata* on three host species: a quantification of assimilate extraction. *Entomol. Exp. Appl.* 107:57Y68.
- BERNAYS, E. A. and WOODHEAD, S. 1984. The need of high levels of phenylalanine in the diet of *Schistocerca gregaria* nymphs. *J. Insect Physiol.* 30:489Y493.
- BI, J. L. and FELTON, G. W. 1995. Foliar oxidative stress and insect herbivory: primary compounds, secondary metabolites and reactive oxygen species as components of induced resistance. *J. Chem. Ecol.* 21:1151Y1530.
- BI, J. L. FELTON, G. W. and MUELLER, A. J. 1994. Induced resistance in soybean to *Helicoverpa zea*: role of plant protein quality. *J. Chem. Ecol.* 20:183Y197.

- BI, J. L. MURPHY, J. B. and FELTON, G. W. 1997. Antinutritive and oxidative components as mechanisms of induced resistance in cotton to *Helicoverpa zea*. *J. Chem. Ecol.* 23:97Y117.
- BI, J. L. BALLMER, G. R. HENDRIX, D. L. HENNEBERRY, T. J. and TOSCANO, N. C. 2001. Effect of cotton nitrogen fertilization on *Bemisia argentifolii* densities and honeydew production. *Entomol. Exp. Appl.* 99:25Y36.
- BI, J. L. TOSCANO, N. C. and MADORE, M. A. 2003. Effect of cotton nitrogen fertilization on *Bemisia argentifolii* populations in relation to soluble proteins and free amino acids in cotton. *J. Chem. Ecol.* 29:747Y761.
- BLACKMER, J. L. and BYRNE, D. N. 1999. Changes in amino acids in *Cucumis melo* in relation to life-history traits and flight propensity of *Bemisia tabaci*. *Entomol. Exp. Appl.* 93:29Y40.
- BLUA, M. J., PHILIPS, P. A. and REDAK, R. A. 1999. A new sharpshooter threatens both crops and ornamentals. *Calif. Agr.* 53:22Y25.
- BRADFORD, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Chem.* 72:248Y254.
- BRODBECK, B. V. and STRONG, D. 1987. Amino acid nutrition of herbivorous insects and stress to host plants, pp. 347Y364, in P. Barbosa and J. C. Schultz (eds.). *Insect Outbreaks*. Academic Press, New York.
- BRODBECK, B. V. MIZELL, R. F. III, FRENCH, W. J. ANDERSON, P. C. and ALDRICH, J. H. 1990. Amino acids as determinants of host preference for the xylem feeding leafhopper, *Homalodisca coagulata* (Homoptera: Cicadellidae). *Oecologia* 83:338Y345.
- BRODBECK, B. V. MIZELL, R. F. III, and ANDERSON, P. C. 1993. Physiological and behavioral adaptations of three species of leafhoppers in response to the dilute nutrient content of xylem fluid. *J. Insect Physiol.* 39:73Y81.
- BRODBECK, B. V. ANDERSON, P. C. and MIZELL, R. F. III. 1995. Differential utilization of nutrients during development by the xylophagous leafhopper, *Homalodisca coagulata*. *Entomol. Exp. Appl.* 75:279Y289.
- BRODBECK, B. V. ANDERSON, P. C. and MIZELL, R. F. III. 1996. Utilization of primary nutrients by the polyphagous xylophage, *Homalodisca coagulata*, reared on single host species. *Arch. Insect Biochem. Physiol.* 32:65Y83.
- BRODBECK, B. V. ANDERSON, P. C., and MIZELL, R. F. III. 1999. Effect of total dietary nitrogen and nitrogen form on the development of xylophagous leafhoppers. *Arch. Insect Biochem. Biophysiol.* 42:37Y50.
- BRODBECK, B. V., ANDERSON, P. C., MIZELL, R. F. III, and ODEN, S. 2004. Comparative nutrition and developmental biology of xylem-feeding leafhoppers reared on four genotypes of *Glycine max*. *Environ. Entomol.* 33:165Y173.
- CASTLE, S. J., BYRNE, F. J., BI, J. L. and TOSCANO, N. C. 2005. Spatial and temporal distribution of imidacloprid and thiamethoxam in citrus and impact on *Homalodisca coagulata* populations. *Pest Manag. Sci.* 61:75Y84.
- FELTON, G. W. 1996. Nutritive quality of plant protein: sources of variation and insect herbivore responses. *Arch. Insect Biochem. Physiol.* 32:39Y54.
- HIX, R. L. 2001. Egg-laying and brochosome production observed in glassy-winged sharpshooter. *Calif. Agric.* 53:19Y22.
- JOERN, A. and BEHMER, S. T. 1997. Importance of dietary nitrogen and carbohydrates to survival, growth, and reproduction in adults of the grasshopper *Ageneotettix deorum* (Orthoptera: Acrididae). *Oecologia* 112:201Y208.
- LITTLE, T. M. and HILL, F. J. 1978. *Agricultural Experimentation V Design and Analysis*. Wiley, New York, NY.
- MATTSON, W. J. 1980. Herbivory in relation to plant nitrogen content. *Annu. Rev. Ecol. Syst.* 11:119Y161.
- MCNEILL, S. and SOUTHWOOD, T. R. 1978. The role of nitrogen in the development of insect plant

- relationships, pp. 77Y98, in J. Harborne (ed.). *Biochemical Aspects of Plant and Animal Coevolution*. Academic Press, London.
- MIZELL, R. F. and FRENCH, W. J. 1987. Leafhopper vectors of phony peach disease: Feeding site preference and survival on infected and uninfected peach, and seasonal response to selected host plants. *J. Entomol. Sci.* 22:11Y22.
- MORENO, J. and GARCIA-MARTINEZ, J. L. 1983. Seasonal variation of nitrogenous compounds in the xylem sap of Citrus. *Physiol. Plant.* 59:669Y675.
- OHGUSHI, T. 1992. Resource limitation on insect herbivore populations, pp. 199Y241, in M. D. Hunter, and P. W. Price (eds.). *Effects of Resource Distribution on AnimalYPlant Interactions*. Academic Press, San Diego.
- PERRING, T. M. FARRAR, C. A., and BLUA, M. J. 2001. Proximity to citrus influences Pierce's disease in Temecula Valley vineyards. *Calif. Agric.* 55:13Y18.
- PURCELL, A. H. 1981. Pierce's disease, pp. 62Y69, in D. L. Flaherty, F. L. Jenson, and A. N. Kasamatis (eds.). *Grape Pest Management*. Division of Agricultural Science, No. 4105. UC, Berkeley.
- REDAK, R. A., PURCELL, A. H., LOPES, J. R. S., BLUA, M. J., and MIZELL, R. F. III. 2004. The biology of xylem fluid-feeding insect vectors of *Xylella fastidiosa* and their relation to disease epidemiology. *Annu. Rev. Entomol.* 49:243Y270.
- SAS INSTITUTE INC. 1989. *SAS/STAT User's Guide*, Version 6, Cary, NC.
- SLANSKY, F. JR., 1993. Nutritional ecology: the fundamental quest for nutrients, pp. 29Y91, in N. E. Stamp and T. M. Casey (eds.). *CaterpillarsVEcological and Evolutionary Constraints on Foraging*. Chapman and Hall, New York.
- SORENSEN, J. T. and GILL, R. J. 1996. A range expansion of *Homalodisca coagulata* (Say) (Hemiptera: Clypeorrhncha: Cicadellidae) to Southern California. *Pan-Pacific Entomol.* 72:160Y161.
- SIMPSON, S. J., ABISGOLD, J. D., and DOUGLAS, A. E. 1995. Response of the pea aphid (*Acyrthosiphon pisum*) to variation in dietary levels of sugar and amino acids: the significance of amino acid quality. *J. Insect Physiol.* 41:71Y75.
- TAYLOR, M. W. and MEDICI, J. C. 1966. Amino acid requirements of grain beetles. *J. Nutr.* 88:176Y180.
- TURNER, M. W. and POLLARD, H. N. 1959. Life and histories and behavior of five insect vectors of phony peach disease. *USDA Tech. Bul.* 1188.
- VARELA, L. G. 1996. *Pierce's Disease in the North Coast*. University of California Cooperative Extension & Statewide IPM Project: 1Y11.
- WHITE, T. C. R. 1984. The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia* 63:90Y105.